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Thomas Krahn et al.
Application No. 09/966,522
Amendment Under 37 CFR § 1.111
Reply to Office Action of October 1, 2004

AMENDMENTS TO THE CLAIMS:

This listing of claims will replace all prior versions, and listings, of claims in the application:

Listing of Claims

1. – 5. (Canceled).

6. (Withdrawn) A method of reducing non-specific background light from a biomedical assay comprising: contacting said biomedical assay with a masking dye, which reduces non-specific background light, wherein said masking dye is substantially membrane impermeant; wherein said biomedical assay comprises biological cells on a solid support; wherein said biomedical assay comprises a fluorescent dye, which has membrane permeability; and wherein the fluorescent dye and the masking dye are in an aqueous solution that contacts said biological cells.

7. (Withdrawn) The method of claim 6, wherein the masking dye has an absorption spectra that overlaps with the emission and/or excitation spectrum of the fluorescent dye.

8. (Withdrawn) The method of claim 6, wherein the fluorescent dye is a membrane potential-sensitive dye.

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9. (Withdrawn) The method of claim 7, wherein said masking dye is present in said aqueous solution at a concentration sufficient to reduce light emitted from said aqueous solution by at least 10% compared to the light emitted of said aqueous solution in the absence of said masking dye.
10. (Withdrawn) The method of claim 7, wherein said masking dye is present in said aqueous solution at a concentration sufficient to reduce light emitted from said aqueous solution by at least 70% compared to the light emitted of said aqueous solution in the absence of said masking dye.
11. (Withdrawn) The method of claim 6, wherein said masking dye is non-toxic to said biological cell.
12. (Withdrawn) The method of claim 6, wherein said masking dye is present in said aqueous solution at a concentration of at least 5 mM.
13. (Withdrawn) The method of claim 6, wherein said masking dye is Brilliant Black (tetrasodium 4-acetamido-5-hydroxy-6-[7-sulfonato-4-(4-sulfonatophenylazo)-1-naphthylazo] naphthalene-1,7-disulfonate).
14. (Withdrawn) A method of reducing non-specific background light from a biomedical assay comprising:

contacting said biomedical assay with a masking dye,

wherein said biomedical assay comprises biological cells,

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wherein said biomedical assay comprises a fluorescent dye, which has membrane permeability and which is in contact with said biological cells;

wherein said masking dye is present in said biomedical assay at a concentration, that reduces non-specific background light from said biomedical assay by at least 30% compared to the light emission from said biomedical assay in the absence of said masking dye,

wherein said masking dye is substantially membrane impermeant;

wherein said masking dye does not specifically bind to said biological cell.

15. (Withdrawn) A method of claim 14, wherein said non-specific background light is derived from solution fluorescence.

16. (Withdrawn) A method of claim 14, wherein said masking dye has an absorption spectra that overlaps with the emission and/or excitation spectrum of said fluorescent dye.

17. (Previously Presented) A kit for performing a biomedical assay, comprising:

a) a fluorescent dye; and

b) a masking dye,

wherein said masking dye is substantially impermeant to the membrane of a biological cell,

wherein said masking dye has an absorption spectrum that overlaps with the emission and/or excitation spectrum of said fluorescent dye,

wherein said masking dye does not specifically bind to said membrane of said biological cell, and

wherein said masking dye is present in a solution at an amount sufficient to reduce nonspecific background light emitted from said solution by at least 10% compared to the non-specific

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background light emitted from said solution in the absence of said masking dye.

18. (Previously Presented) The kit of claim 17, wherein said masking dye is present in said solution at an amount sufficient to reduce non-specific background light from said solution by at least 30% compared to the non-specific background light emitted from said solution in the absence of said masking dye.

19. (Previously Presented) The kit of claim 17, wherein said masking dye is present in said solution at an amount sufficient to reduce non-specific background light from said solution by at least 50% compared to the non-specific background light emitted from said solution in the absence of said masking dye.

20. (Previously Presented) The kit of claim 17, wherein said masking dye is present in said solution at an amount sufficient to reduce non-specific background light from said solution by at least 70% compared to the non-specific background light emitted from said solution in the absence of said masking dye.

21. (Previously Presented) The kit of claim 17, wherein said fluorescent dye is permeant to the membrane of said biological cell and detects a voltage across the membrane of said biological cell.

22. (Currently Amended) The kit of claim 17, wherein said ~~fluorescent~~ **masking** dye comprises Brilliant Black (tetrasodium 4-acetamido-5-hydroxy-6-[7-sulfonato-4-(4-sulfonatophenylazo)-1-naphthylazo] naphthalene-1,7-disulfonate).

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23. (Previously Presented) The kit of claim 17, wherein said masking dye improves the optical signal-to-noise-ratio by at least 300% compared to the optical signal-to-noise-ratio of said biomedical assay in the absence of said masking dye.
24. (Withdrawn) A composition of matter, comprising:
- a) a biological cell in contact with a solid surface, wherein said biological cell is in contact with a fluorescent dye, wherein said fluorescent dye is permeant to the membrane of said biological cell,
 - b) an aqueous solution with a masking dye, wherein said aqueous solution is in contact with the membrane of said biological cell,
- wherein said masking dye is substantially impermeant to said membrane of said biological cell,
- wherein said masking dye has an absorption spectrum that overlaps with the emission and/or excitation spectrum of said fluorescent dye, and
- wherein said masking dye is present in said aqueous solution at a concentration sufficient to reduce non-specific background light emitted from said solution by at least 10% compared to non-specific background light emitted from said aqueous solution in the absence of said masking dye.
25. (Withdrawn) The composition of matter of claim 24, wherein said masking dye is present in said aqueous solution at a concentration sufficient to reduce light emitted from said fluorescent

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dye in said aqueous solution by at least 30% compared to the light emitted from said fluorescent dye in said aqueous solution in the absence of said masking dye.

26. (Withdrawn) The composition of matter of claim 24, wherein said masking dye is present in said aqueous solution at a concentration sufficient to reduce light emitted from said fluorescent dye in said aqueous solution by at least 70% compared to the light emitted from said fluorescent dye in said aqueous solution in the absence of said masking dye.

27. (Withdrawn) The composition of matter of claim 24, wherein said masking dye has an absorption spectrum that overlaps with the emission and/or excitation spectrum of said fluorescent dye.

28. (Withdrawn) The composition of matter of claim 24, wherein said composition further includes a microtitre plate and said biological cell is a member of a plurality of biological cells in a well of said microtitre plate.

29. (Withdrawn) The composition of matter of claim 24, wherein said composition further comprises a system to launch light of a predetermined wavelength through said solid surface the biological cells are in contact with, wherein said predetermined wavelength is an excitation wavelength for said fluorescent dye.

30. (Withdrawn) The composition of matter of claim 24, wherein said masking dye is present in said aqueous solution at a concentration sufficient to reduce non-specific background light emission from said aqueous solution by at least 70% compared to non-specific background light emission in said aqueous solution in the absence of said masking dye.

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31. (Withdrawn) The composition of matter of claim 24, wherein said masking dye is present in said aqueous solution at a concentration of at least 5 mM.
32. (Withdrawn) A method for identifying a chemical with a biological activity, comprising:
- a) contacting a biomedical assay with a test sample,
 - b) contacting said biomedical assay with a masking dye,
- wherein said biomedical assay comprises biological cells in contact with a solid surface, wherein said biomedical assay comprises a fluorescent dye, which has membrane permeability and which is in contact with said biological cells and which directly or indirectly monitors the activity of said cells,
- wherein said masking dye is in an aqueous solution, that contacts the outer surface of the cells, and
- wherein said masking dye has an absorption spectra that overlaps with the emission and/or excitation spectrum of said fluorescent dye,
- wherein said masking dye is substantially impermeant to the membrane of said cells,
- wherein said masking dye does not specifically bind to said cells,
- wherein said masking dye is present in said aqueous solution at a concentration sufficient to reduce non-specific background light from said aqueous solution by at least 10% compared to the light emitted of said aqueous solution in the absence of said masking dye,
- c) detecting an optical signal from said fluorescent dye,

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- d) comparing the optical signal from said fluorescent dye to a separate control signal from a control batch.
33. (Withdrawn) A method of claim 32, wherein said fluorescent dye is a potential sensitive dye and wherein the activity of said cells comprises membrane potential changes.
34. (Withdrawn) A method of claim 32, wherein membrane potential changes below 5 mV can be detected.
35. (Withdrawn) A medical compound identified by a method comprising the steps of:
- a) contacting a biomedical assay with a test sample,
 - b) contacting said biomedical assay with a masking dye,
- wherein said biomedical assay comprises biological cells in contact with a solid surface, wherein said biomedical assay comprises a fluorescent dye, which has membrane permeability and which is in contact with said biological cells and that directly or indirectly monitors the activity of said cells,
- wherein said masking dye is in an aqueous solution, that contacts the outer surface of the cell, and
- wherein said masking dye has an absorption spectra that overlaps with the emission and/or excitation spectrum of said fluorescent dye,
- wherein said masking dye is substantially impermeant to the membrane of said cells,
- c) detecting an optical signal from said fluorescence dye.

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36. (Withdrawn) A method of reducing non-specific background light from a biomedical assay comprising:
- contacting said biomedical assay with a masking dye, which reduces non-specific background light;
- wherein said biomedical assay comprises a receptor layer which is specific for a fluorescent or luminescent ligand in contact with a solid support;
- wherein said biomedical assay comprises fluorescent or luminescent ligands, which are in contact with said receptor layer; and
- wherein the masking dye is in an aqueous solution, that contacts the receptor layer.
37. (Withdrawn) A composition of matter, comprising:
- a) a receptor layer in contact with a solid surface, wherein said receptor is specific for a fluorescent or luminescent ligand, and
- b) an aqueous solution with a masking dye and with fluorescent or luminescent ligands, wherein said aqueous solution is in contact with said receptor layer,
- wherein said masking dye has an absorption spectrum that overlaps with the emission and/or excitation spectrum of said fluorescent ligands,
- and
- wherein said masking dye is present in said aqueous solution at a concentration sufficient to reduce non-specific background light emitted from said solution by at least 10% compared to non-specific background light emitted from said aqueous solution in the

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absence of said masking dye.

38. (Withdrawn) The method according to claim 6, wherein the masking dye comprises dye pigments or inorganic finely divided particles.
39. (Withdrawn) The method according to claim 6, wherein said masking dye has a solubility in water of >2 mg/ml.
40. (Withdrawn) The method according to claim 6, wherein said masking dye is non-toxic to said biological cell.
41. (Withdrawn) The method according to claim 6, wherein said masking dye includes Brilliant Black (tetrasodium 4-acetamido-5-hydroxy-6-[7-sulfonato-4-(4-sulfonatophenylazo)-1-naphthylazo] naphthalene-1,7-disulfonate).
42. The method according to claim 6, wherein the fluorescent dye comprises Dibac₄(3).